SI APPENDIX, Supporting Information to Accompany:

Cryo-EM structure of the bacteriophage T4 isometric head at 3.3Å resolution and its relevance to the assembly of icosahedral viruses

Zhenguo Chen, Lei Sun, Zhihong Zhang, Andrei Fokine, Victor Padilla-Sanchez, Dorit Hanein, Wen Jiang, Michael G. Rossmann, and Venigalla B. Rao

Supporting Information includes:

Figures S1 – S6

Tables S1 – S6

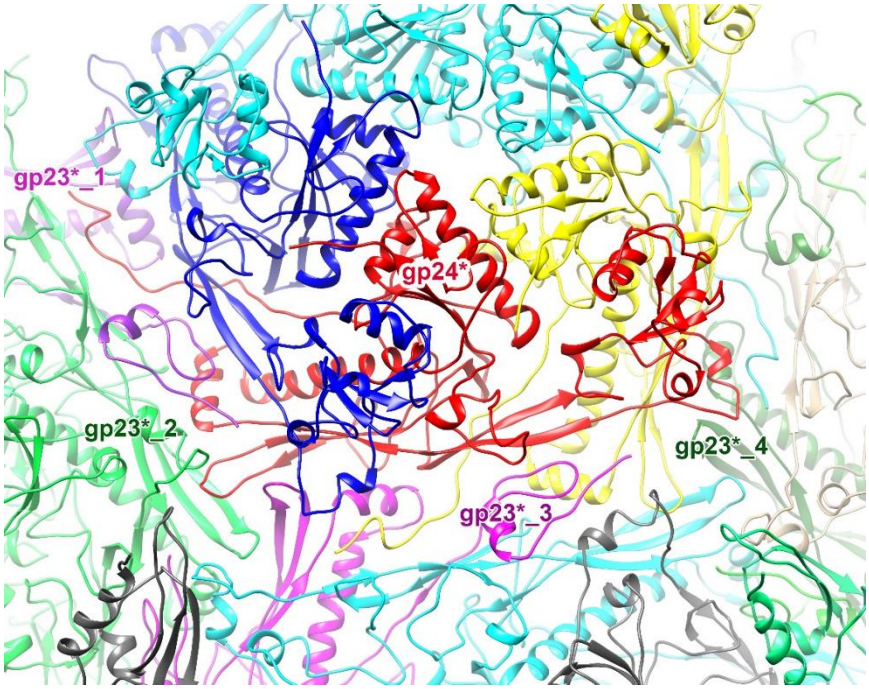

Fig. S1. Interactions of gp24* molecule (red) with other gp24* and gp23* subunits in the capsid. Each molecule is shown as a ribbon diagram in a different color.

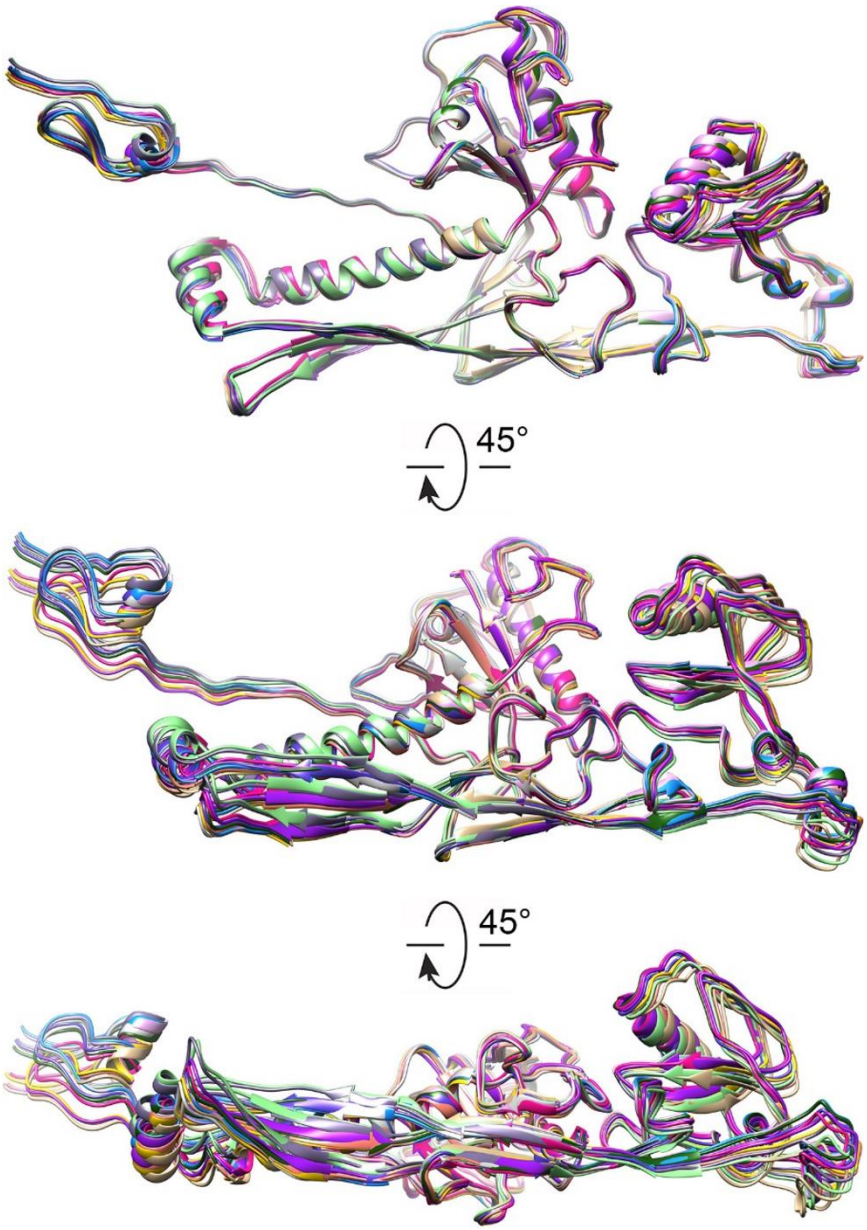

Fig. S2. Superimposition of twelve gp23 molecules within one asymmetric unit. The top figure shows the structure as viewed from the outside of the virus, whereas the bottom figure is a side view.

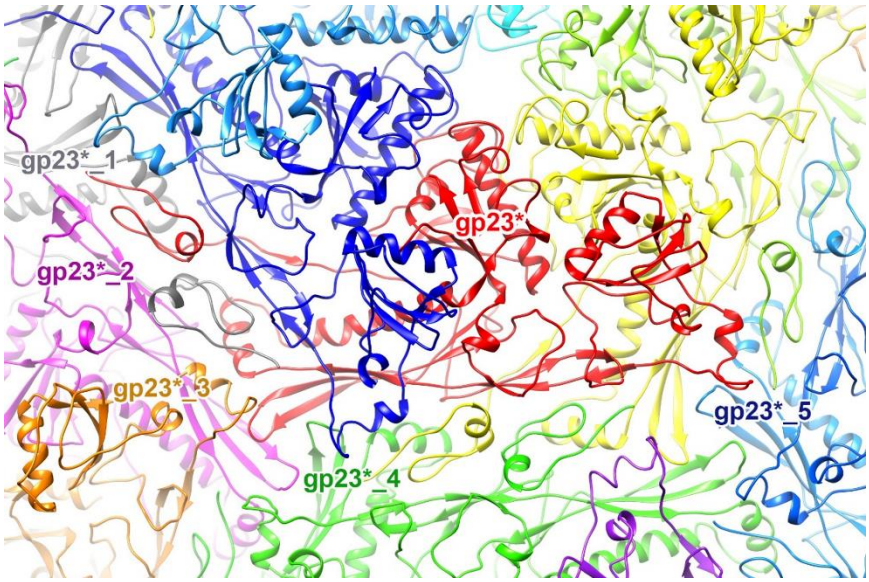

Fig. S3. Interactions of gp23* molecule (red) with five other gp23* molecules belonging to the neighboring capsomers. Each molecule is shown as a ribbon diagram in a different color.

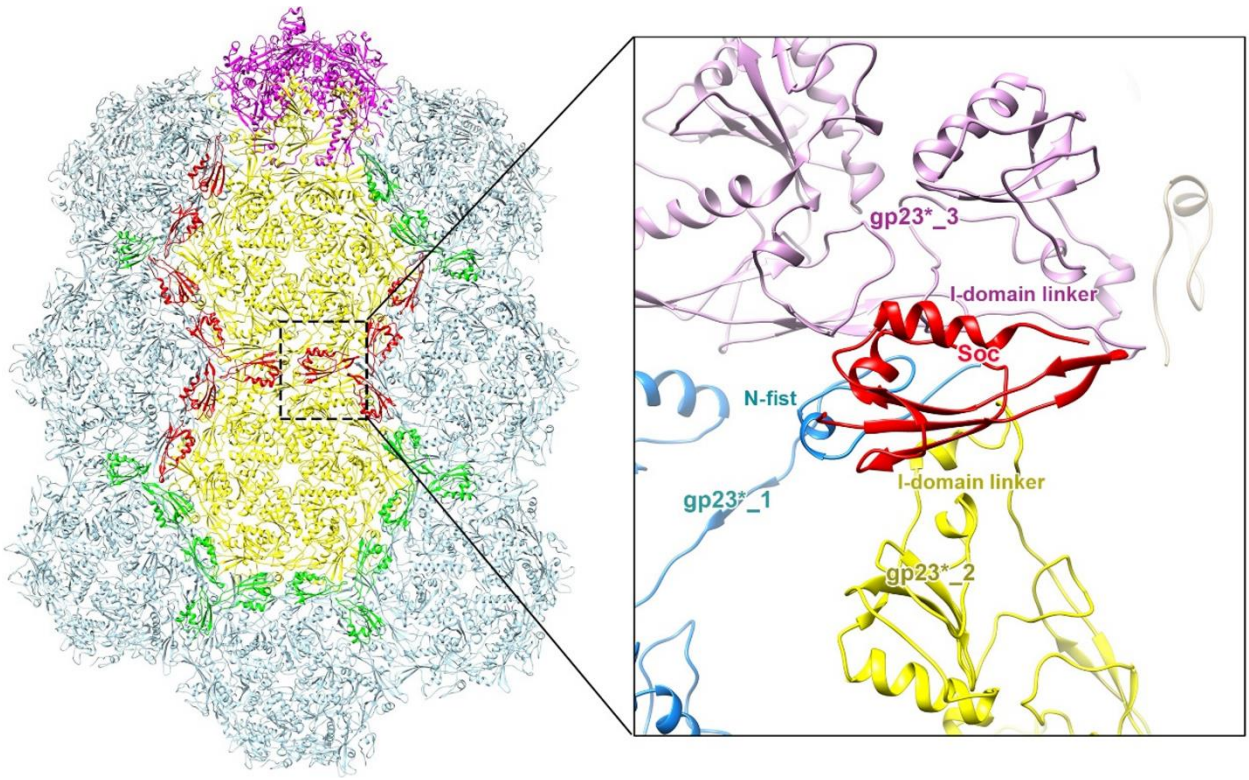

Fig. S4. Interactions of the Soc protein with gp23* molecules in the capsid. (Left panel) Gp23* and gp24* molecules belonging to the icosahedral asymmetric unit are shown in yellow. The Soc molecules belonging to the asymmetric unit are shown in red. The neighboring gp23* hexamers are shown in blue. The gp24* molecules related by the 5-fold symmetry are shown in magenta. Some Soc molecules related by the icosahedral symmetry are shown in green. (Right panel) One Soc molecule (red) interacting with three gp23* molecules (blue, yellow, and light purple). The Soc binding site is made by the N-fist of one gp23* subunit (gp23*_1, blue), an I-domain linker from a second subunit belonging to the same capsomer (gp23*_2, yellow), and an I-domain linker from another subunit belonging to a different capsomer (gp23*_3, light purple).

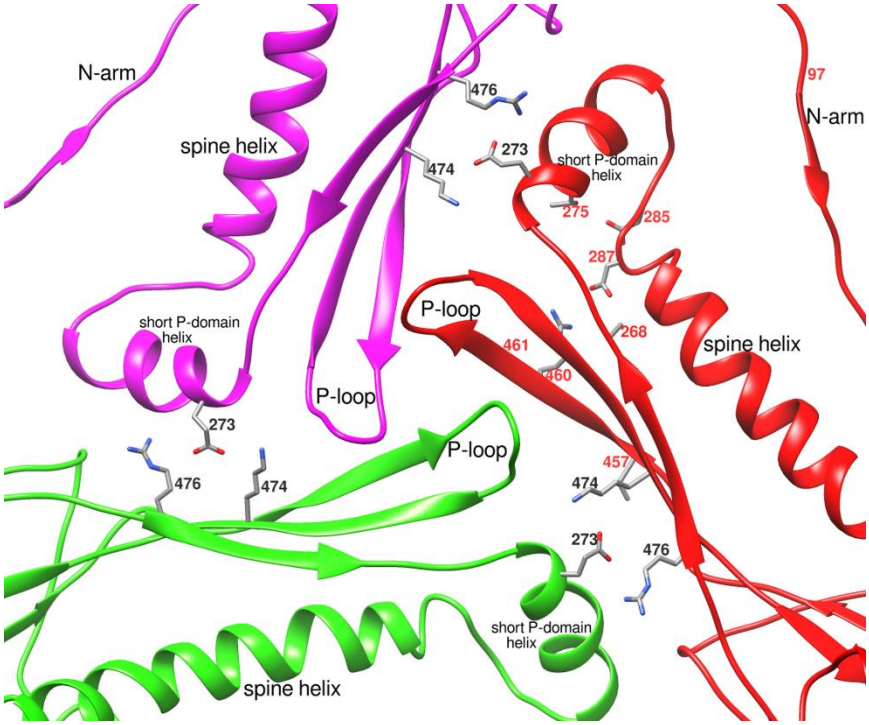

Fig. S5. Interaction of three gp23* molecules (magenta, green, and red) near a quasi-3-fold axis of the capsid. The atomic structure of the side chains for residues Lys^{474}, Arg^{476} and Glu^{273} involved in electrostatic interactions are also shown. Mutation sites that affect the capsid length are indicated by red labels. The side chains of the residues corresponding to the mutation sites are shown.

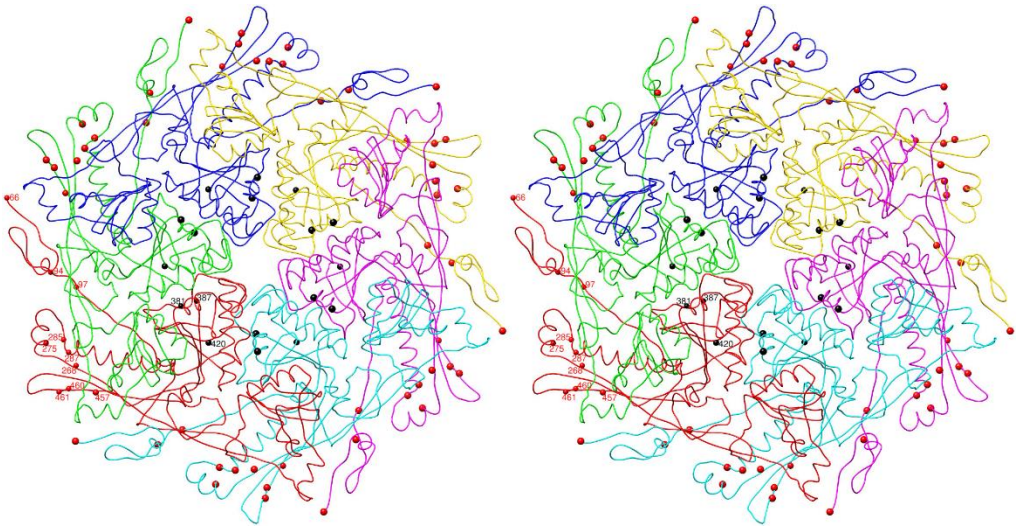

Fig. S6. Stereographic view of a gp23* hexamer showing the positions of mutation sites. The mutation sites that affect the capsid length are depicted by red dots. The gp24-bypass mutation sites are depicted by black dots.

Table S1. Refinement of the isometric T4 head structure.

Resolution (Å)	3.3
Correlation between the experimental and calculated density (across all atoms)	0.80
R.m.s. deviations	
Bond lengths (Å)	0.012
Bond angles (°)	1.041
Ramachandran plot values	
Most favored (%)	90.27
Generously allowed (%)	9.12
Disallowed regions (%)	0.61

Table S2. Superposition of the HK97 major capsid protein onto T4 gp24. Shown are the r.m.s.d. values between equivalenced Cα atoms. The number of equivalence atoms is given in parenthesis.

	Crystal structure of gp24 (uncleaved, immature)	Cryo-EM structure of gp24* (cleaved, mature)
HK97 Head II (mature)	2.9 (192)	2.4 (214)
HK97 prohead II (immature)	2.8 (187)	2.9 (187)

Table S3. Superimposition of gp24* and the 12 gp23* subunits within one asymmetric unit of the icosahedral head. Given are the r.m.s.d. values between equivalenced Cα atoms.

gp23	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
gp24	**2.6**	**2.8**	**2.9**	**2.8**	**2.7**	**2.9**	**3.0**	**2.9**	**3.0**	**2.9**	**2.9**	**2.9**

Table S4. Superimposition of 12 gp23* subunits within one asymmetric unit. Given are the r.m.s.d. values between equivalenced Cα atoms. The smallest r.m.s.d. values between subunits belonging to the hexamer ABCDEF are shown in red. The smallest r.m.s.d. values between subunits belonging to different hexamers are highlighted in yellow.

	A	B	C	D	E	F	G	H	I	J	K	L
A	0	1.7	1.7	1.1	1.5	1.7	1.2	1.7	1.5	1.7	1.7	1.6
B	1.7	0	1.2	1.2	0.8	1.0	1.2	0.2	1.2	0.7	0.7	1.0
C	1.7	1.2	0	1.1	1.3	0.8	1.0	1.2	0.5	1.0	0.9	0.5
D	1.1	1.2	1.2	0	1.1	1.4	0.5	1.2	1.0	1.1	1.2	1.0
E	1.5	0.8	1.3	1.1	0	1.1	0.9	0.8	1.3	0.7	0.8	1.0
F	1.7	1.0	0.8	1.4	1.1	0	1.2	1.0	0.9	0.9	0.8	0.8
G	1.2	1.2	1.0	0.5	0.9	1.2	0	1.2	0.9	0.9	1.1	0.8
H	1.7	0.2	1.2	1.2	0.8	1.0	1.2	0	1.2	0.7	0.7	1.0
I	1.5	1.2	0.5	1.0	1.3	0.9	0.9	1.2	0	1.0	0.9	0.6
J	1.7	0.7	1.0	1.1	0.7	0.9	0.9	0.7	1.0	0	0.3	0.7
K	1.7	0.7	0.9	1.2	0.8	0.8	1.1	0.7	0.9	0.3	0	0.7
L	1.6	1.0	0.5	1.0	1.0	0.8	0.8	1.0	0.6	0.7	0.7	0

Table S5. Occupancies of the Soc molecules.

Angle between adjacent gp23* capsomers (°)	Occupancies of Soc molecules located at the inter-capsomer interface	
33	0.57	0.59
22	0.51	0.51
19	0.43	0.41
18	0.49	0.48
17	0.49	0.49
6	0.42	0.38

Table S6. Deviations of the centers of mass of the gp23* subunits from the mean hexamer planes.

gp23* subunit	Deviation of the center of mass from the mean hexamer plane (Å)
A	**0.78**
B	**0.57**
C	**0.21**
D	**0.79**
E	**0.59**
F	**0.20**
G	**0.26**
H	**0.46**
I	**0.19**
J	**0.28**
K	**0.47**
L	**0.20**